Remarks

Please enter and consider new claims 46-50, and original claims 1-45.

The figures and related description find support in the documents incorporated by reference.

The Examiner is invited to contact Applicants' Representatives at the below-listed telephone number, if there are any questions regarding this Preliminary Amendment or if prosecution of this application may be assisted thereby.

Respectfully submitted for

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APPENDIX A - SPECIFICATION/CLAIM AMENDMENTS INCLUDING NOTATIONS TO INDICATE CHANGES MADE
Serial No.: 09/841,264
Docket No.: 56286US003

Amendments to the following are indicated by underlining what has been added and bracketing what has been deleted. Additionally, all amendments have been marked in bold.

In the Specification

The following paragraphs are inserted after the paragraph beginning at page 6, line 10.

FIG. 2 is a top plan view of one device according to the present invention.

FIG. 3 is a top plan view of another device according to the present invention.

FIG. 4 is a cross-sectional view of the device of FIG. 3 taken along line 4-4 in FIG. 3.

The paragraph beginning at page 13, line 26, has been amended as follows:

Although the methods can be used in a variety of devices, a variety of illustrative embodiments of preferred devices are described in U.S. Patent Application Serial No. 60/214,508 filed on June 28, 2000 and entitled THERMAL PROCESSING DEVICES AND METHODS. Other useable device constructions may be found in, e.g., U.S. Patent Application Serial No. 09/710,184 filed on November 10, 2000 and entitled CENTRIFUGAL FILLING OF SAMPLE PROCESSING DEVICES**, as well as U.S. Provisional Patent Application Serial No. 60/214,642 filed on June 28, 2000 and entitled SAMPLE PROCESSING DEVICES, SYSTEMS AND METHODS and U.S. Provisional Patent Application Serial No. 60/237,072 filed on October 2, 2000 and entitled SAMPLE PROCESSING DEVICES, SYSTEMS AND METHODS.**

The paragraph beginning at page 14, line 1, has been amended as follows:

Regardless of the specific device, a fluid sample (e.g., solution) in a process chamber can be interrogated by electromagnetic energy of selected wavelengths (if desired). Suitable electromagnetic energy is supplied by an electromagnetic energy source that directly heats the fluid (e.g., solution) in the process chamber source and is preferably remote from the device, i.e., it is not located on the device. Examples of some suitable electromagnetic energy sources may include, but are not limited to, lasers, broadband electromagnetic energy sources (e.g., white light), etc. The electromagnetic energy source may be provided continuously or intermittently based on a variety of factors, e.g., the desired temperature of the sample materials, the rate at which thermal energy is removed from each process chamber, the desired rate of temperature change, whether the process chambers include a reflective component, etc. If the electromagnetic energy source is cycled or otherwise varied, a registration system may be used to deliver a selected amount of energy to selected process chambers and an optional additional temperature control mechanism in the form of a fluid source, e.g., pressurized air or any other suitable fluid, can be directed at the surface of the device. **[These are discussed in U.S. Patent Application Serial No. 60/214,508 filed on June 28, 2000 and entitled THERMAL PROCESSING DEVICES AND METHODS.]**

The paragraph beginning at page 16, line 27, has been amended as follows:

A preferred method involves the use of a device with a plurality of process chambers and/or process chamber arrays such as those illustrated and described in U.S. Patent Application Serial No. **[60/214,508]** **60/214,642** filed on June 28, 2000 and entitled **[THERMAL PROCESSING DEVICES AND METHODS]** **SAMPLE PROCESSING DEVICES, SYSTEMS AND METHODS**. Each of the devices described in that application

include a number of chambers that are preferably arranged generally radially on a device (such that centrifugal forces can move fluids within the devices). The chambers are in fluid communication using channels or other conduits that may, in some embodiments, include valve structures to control the movement as desired.

Please insert the following paragraphs before the paragraph beginning at page 18, line 1:

The present invention also provides devices for thermal processing of sample materials. The sample materials may be located in a plurality of process chambers in the device which, in various aspects, may include one or more of: a reflective layer (e.g., a metallic layer); baffle structures to enhance cooling during rotation of the device; capture plugs to capture filtering materials; valve mechanisms capable of being selectively opened, thermal indicators for monitoring/controlling the temperatures in process chambers, absorptive materials in the process chambers to enhance energy absorption, etc. In various embodiments, the devices may include reagents, filters, and other sample processing materials in the process chambers.

Among the thermal control advantages of the devices of the present invention are chamber-to-chamber temperature uniformity, comparable chamber-to-chamber temperature transition rates, and the increased speed at which thermal energy can be added or removed from the process chambers. Among the device features than can contribute to these thermal control advantages are the inclusion of a reflective layer (e.g., metallic) in the device, baffle structures to assist in removing thermal energy from the device, and low thermal mass of the device. By including thermal indicators in the devices, enhanced control over chamber temperature may be achieved even as the device is rotated during processing.

One illustrative device manufactured according to the principles of the present invention is illustrated in Figure 2. The device 10 is preferably in the shape of a circular disc as illustrated in Figure 1, although any other shape than can be rotated could be used in place of the preferred circular disc.

The device includes a plurality of process chambers 50, each of which defines a volume for containing a sample and any other materials that are to be thermally cycled with the sample. The illustrated device 10 includes ninety-six process chambers 50, although it will be understood that the exact number of process chambers provided in connection with a device manufactured according to the present invention may be greater than or less than ninety-six, as desired.

The process chambers 50 in the illustrative device 10 are in the form of chambers, although the process chambers in devices of the present invention may be provided in the form of capillaries, passageways, channels, grooves, or any other suitably defined volume.

The process chambers 50 are in fluid communication with distribution channels 60 that, together with loading port 62, provide a distribution system for distributing samples to the process chambers 50. Introduction of samples into the device 10 through the loading port 62 may be accomplished by rotating the device 10 about a central axis of rotation such that the sample materials are moved outwardly due to centrifugal forces generated during rotation. Before the device 10 is rotated, the sample can be introduced into the loading port 62 for delivery to the process chambers 50 through distribution channels 60. The process chambers 50 and/or distribution channels 60 may include ports through which air can escape and/or features to assist in distribution of the sample materials to the process chambers 50. Alternatively, it may be possible to provide a closed distribution system, i.e., a system in which materials may be introduced through an opening through which air within the process chambers 50 and/or distribution channels 60 also escapes during the distribution process. In

another alternative, sample materials could be loaded into the process chambers 50 under the assistance of vacuum or pressure.

The illustrated device 10 includes a loading port 62 with two chambers 64 that are isolated from each other. As a result, a different sample can be introduced into each chamber 64 for loading into the process chambers 50 that are in fluid communication with the respective chamber 64 of the loading port 62 through distribution channels 60. It will be understood that the loading port 62 may contain only one chamber or that any desired number of chambers 64, i.e., two or more chambers 64, could be provided in connection with the device 10.

Figures 3 & 4 illustrate another embodiment of a device and methods according to the present invention. The device 110 includes a number of sets of interconnected process chambers that can be described as process chamber arrays in connection with the present invention. The device 110 includes a first cover layer 130 attached to a first major side 122 of the substrate 120 and a second cover layer 140 attached to a second major side 124 of the substrate 120. The substrate 120 and cover layers 130 and 140 may be attached by any suitable technique or techniques, including, but not limited to, adhesives, welding (chemical and/or thermal), etc.

The first cover layer 130 may be homogeneous or it may include multiple sub-layers. It may be preferred that the first cover layer 130 be reflective for electromagnetic energy of selected wavelengths as described above. The second cover layer 140 may include, e.g., an adhesive on a carrier layer, both of which may be optically clear or otherwise transmissive to electromagnetic energy of selected wavelengths.

Among the features formed in the substrate 120 are a loading chamber 160 that, in the illustrated embodiment, is in the form of an annular ring. Each of the process chamber arrays also includes inner or first process chambers 150a and outer or second process chambers 150b located further out radially from a center of the device 110.

The loading chamber 160 is in fluid communication with the inner process chamber 150a through channel 162. As a result, rotation of the device 110 about its center will force sample material to move from the loading chamber 160 into the first process chamber 150a where the first thermal processing of the sample material may be performed.

The process chamber arrays also include a valve 170 located between and separating the pair of inner and outer process chambers 150a and 150b. The valve 170 is normally closed when the device 110 is supplied to a user to prevent movement of the sample material from the first process chamber 150a into the second process chamber 150b.

The valve 170 may preferably be located within a via 180 that is in fluid communication with inner process chamber 150a through channel 182 on one side and in fluid communication with the outer process chamber 150b through channel 184 on the opposite side. It may be preferred that the via 180 be formed such that it extend between the first and second major surfaces 122 and 124 of the substrate 120 as depicted.

The valve 170 includes an impermeable disc 172 that prevents fluids from moving between the process chambers 150a and 150b when it is intact. The impermeable disc 172 is preferably distinct from the substrate 120, i.e., it is preferably made of a material that is different than the material used for the substrate 120. By using different materials for the substrate 120 and the impermeable disc 172, each material can be selected for its desired characteristics.

The impermeable disc 172 may be made of any suitable material, although it may be preferred that the material of the disc 172 form voids without the production of any significant byproducts, waste, etc. that could interfere with the reactions or processes taking place in process chambers. A preferred class of materials are pigmented oriented polymeric films, such as, for example, films used to manufacture commercially available can liners or bags. A suitable film may be a black can liner, 1.18 mils thick, available from Himolene Incorporated, of Danbury, Connecticut under the designation 406230E.

It may further be preferred that the impermeable disc 172 of the valve 170 include material susceptible of absorbing electromagnetic energy of selected wavelengths and converting that energy to heat, resulting in the formation of a void in the impermeable disc 172. The absorptive material may be contained within the impermeable disc 172 or coated on a surface thereof.

The valve 170 illustrated in Figure 4 also includes an optional permeable support 174 located proximate at least one side of the impermeable disc 172. The support 174 is permeable to the fluids moving between the process chambers 150a and 150b, although it may perform some filtering functions in addition to supporting the impermeable disc 172. It may be preferred that the support 174 be somewhat resilient to assist in sealing the valve 170 by forcing the impermeable disc 172 against the surfaces in the via 180 with sufficient force to prevent fluid passage in ordinary use of the device 110.

It may be preferred that the support 174 be provided in the form of a porous disc as illustrated in Figure 4. The porous disc support 174 may preferably be coextensive with the impermeable disc 172 used in the valve 170. Alternative forms of the support may include rings, sleeves, or any other structure or material that can support at least a portion of the impermeable disc 172 in the valve 170.

In some embodiments, it may be desirable that the porous disc support 174 reflect electromagnetic energy of selected wavelengths to assist in the opening of the valve 170 and/or prevent the electromagnetic energy from reaching any underlying fluids, sample materials, etc.

It may be preferred that the porous disc support 174 be hydrophobic to reduce or prevent fluid contact with the impermeable disc 172. Alternatively, it may be preferred that the porous disc support 174 be hydrophilic to promote fluid contact with the impermeable disc 172 of the valve 170.

Examples of suitable materials for a porous disc support may include, but are not limited to, porous plugs or membranes, including scintered polypropylene and scintered

polyethylene plugs or membranes, e.g., such as those commercially available from Porex Corporation, Fairburn, Georgia.

The valve 170 is opened by forming a void in the impermeable disc 172. The void may be formed by electromagnetic energy of any suitable wavelength. It may be preferred that laser energy of a suitable wavelength be used. A potential advantage of using laser energy is that the same laser used to heat the materials in the process chambers may be used to form the voids needed to place the process chambers in fluid communication with each other.

It may further be desirable to place the impermeable disc 172 of the valve 170 within a via 180 as illustrated in Figure 4. Locating the impermeable disc 172 within a via 180 and directing electromagnetic energy of some wavelengths into the via 180 may result in some advantages in that the walls of the via 180 may reflect and/or focus at least some of the energy to assist in formation of the void in the disc 172.

In the Claims

For convenience, all pending claims are shown below.

1. A composition comprising an enzyme, a dye, and an effective amount of a surfactant selected from the group of a nonionic surfactant, a zwitterionic surfactant, and a mixture thereof, wherein the dye inactivates the enzyme in the absence of the surfactant, and the surfactant inhibits such inactivation.

2. The composition of claim 1 wherein the dye is selected from the group of a near-IR dye, a uv/visible dye, a fluorescent dye, and a mixture thereof.

3. The composition of claim 2 wherein the dye is a near-IR dye.

4. The composition of claim 3 wherein the near-IR dye is a diimminium dye or a cyanine dye.

5. The composition of claim 1 wherein the enzyme is a polymerase or a ligase.

6. The composition of claim 1 wherein the nonionic surfactant is selected from the group of esters of fatty acids and polyhydric alcohols, fatty acid alkanolamides, ethoxylated fatty acids, ethoxylated aliphatic acids, ethoxylated fatty alcohols, ethoxylated aliphatic alcohols, ethoxylated sorbitol fatty acid esters, ethoxylated glycerides, ethoxylated block copolymers with EDTA, ethoxylated cyclic ether adducts, ethoxylated amide and imidazoline adducts, ethoxylated amine adducts, ethoxylated mercaptan adducts, ethoxylated condensates with alkyl phenols, ethoxylated nitrogen-based hydrophobes, ethoxylated polyoxypropylenes, polymeric silicones, fluorinated surfactants, polymerizable surfactants, and mixtures thereof.

7. The composition of claim 1 wherein the zwitterionic surfactant is selected from the group of alkylamido betaines and amine oxides thereof, alkyl betaines and amine oxides thereof, sulfo betaines, hydroxy sulfo betaines, amphoglycinates, amphopropionates, balanced amphopolycarboxyglycinates, and alkyl polyaminoglycinates, and mixtures thereof.

8. The composition of claim 1 wherein the dye is present at a concentration of at least about 0.005 mg/mL.

9. The composition of claim 1 wherein the effective amount of surfactant is at least about 0.5 wt-%.

10. The composition of claim 9 wherein the effective amount of surfactant is no greater than about 20 wt-%.

11. The composition of claim 1 further comprising a buffer.

12. The composition of claim 1 further comprising a dinucleotide triphosphate.

13. The composition of claim 1 further comprising a reference dye.

14. The composition of claim 1 further comprising an antioxidant.

15. The composition of claim 14 wherein the dye is capable of optical degradation.

16. The composition of claim 1 wherein the surfactant is an antioxidant.

17. A composition comprising a polymerase enzyme, a near-IR dye, and an effective amount of a surfactant selected from the group of a nonionic surfactant, a zwitterionic surfactant, and a mixture thereof, wherein the near-IR dye inactivates the enzyme in the absence of the surfactant, and the surfactant inhibits the inactivation.

18. A composition comprising:

a polymerase enzyme;

a near-IR dye selected from the group of a diimminium dye, a cyanine dye, and a mixture thereof; and

an effective amount of a nonionic surfactant;

wherein the near-IR dye inactivates the enzyme in the absence of the surfactant, and the surfactant inhibits the inactivation.

19. A composition comprising:

a polymerase enzyme;

a near-IR dye selected from the group of a diimminium dye, a cyanine dye, and a mixture thereof; and

an effective amount of a nonionic surfactant selected from the group of esters of fatty acids and polyhydric alcohols, fatty acid alkanolamides, ethoxylated fatty acids, ethoxylated aliphatic acids, ethoxylated fatty alcohols, ethoxylated aliphatic alcohols, ethoxylated sorbitol fatty acid esters, ethoxylated glycerides, ethoxylated block copolymers with EDTA, ethoxylated cyclic ether adducts, ethoxylated amide and imidazoline adducts, ethoxylated amine adducts, ethoxylated mercaptan adducts, ethoxylated condensates with alkyl phenols, ethoxylated nitrogen-based hydrophobes, ethoxylated polyoxypropylenes, polymeric silicones, fluorinated surfactants, polymerizable surfactants, and mixtures thereof;

wherein the near-IR dye inactivates the enzyme in the absence of the surfactant, and the surfactant inhibits the inactivation.

20. A method of stabilizing an enzyme in a fluid sample in the presence of a dye under conditions that normally inactivate the enzyme, the method comprising combining an effective amount of a surfactant selected from the group of a nonionic surfactant, a zwitterionic surfactant, and a mixture thereof, with the enzyme and the dye, wherein the surfactant inhibits inactivation of the enzyme.

21. The method of claim 20 wherein the dye is selected from the group of a near-IR dye, a uv/visible dye, a fluorescent dye, and a mixture thereof.

22. The method of claim 21 wherein the enzyme is a polymerase or a ligase.

23. The method of claim 20 wherein the surfactant is a nonionic surfactant selected from the group of esters of fatty acids and polyhydric alcohols, fatty acid alkanolamides, ethoxylated fatty acids, ethoxylated aliphatic acids, ethoxylated fatty alcohols, ethoxylated aliphatic alcohols, ethoxylated sorbitol fatty acid esters, ethoxylated glycerides, ethoxylated block copolymers with EDTA, ethoxylated cyclic ether adducts, ethoxylated amide and imidazoline adducts, ethoxylated amine adducts, ethoxylated mercaptan adducts, ethoxylated condensates with alkyl phenols, ethoxylated nitrogen-based hydrophobes, ethoxylated polyoxypropylenes, polymeric silicones, fluorinated surfactants, polymerizable surfactants, and mixtures thereof.

24. A method of stabilizing a polymerase enzyme in solution in the presence of a near-IR dye under conditions that normally inactivate the enzyme, the method comprising combining an effective amount of a nonionic surfactant with the enzyme and the dye, wherein the surfactant inhibits inactivation of the enzyme.

25. A method of conducting a thermal process, the method comprising:

providing a sample mixture comprising a biological material, an enzyme, an effective amount of a surfactant selected from the group of a nonionic surfactant, a zwitterionic surfactant, and a mixture thereof, and a dye at a first temperature; and

directly heating the sample mixture to a second temperature higher than the first temperature;

wherein the dye inactivates the enzyme in the absence of the surfactant and the surfactant inhibits the inactivation.

26. The method of claim 25 further comprising cooling the sample mixture and directly reheating the sample mixture in a thermal cycling process.

27. The method of claim 26 wherein the thermal cycling process comprises at least about 25 cycles.

28. The method of claim 27 wherein the first temperature is within a range of about 0°C to about 50°C.

29. The method of claim 27 wherein the second temperature is within a range of about 50°C to about 95°C.

30. The method of claim 27 wherein the thermal cycling process comprises heating between a temperature of about 50°C and about 95°C.

31. A method of conducting a thermal cycling process, the method comprising:

providing a device comprising at least one process chamber that defines a volume for containing a sample mixture comprising a biological material, an enzyme, a dye, and a surfactant selected from the group of a nonionic surfactant, a zwitterionic surfactant, and a mixture thereof;

delivering electromagnetic energy to the process chamber to raise the temperature of the sample material in the process chamber, wherein the dye converts the electromagnetic energy into thermal energy;

wherein the surfactant inhibits interaction between the enzyme and the dye.

32. The method of claim 31 wherein the dye is a near-IR dye.

33. The method of claim 31 wherein the surfactant is a nonionic surfactant selected from the group of esters of fatty acids and polyhydric alcohols, fatty acid alkanolamides, ethoxylated fatty acids, ethoxylated aliphatic acids, ethoxylated fatty alcohols, ethoxylated aliphatic alcohols, ethoxylated sorbitol fatty acid esters, ethoxylated glycerides, ethoxylated block copolymers with EDTA, ethoxylated cyclic ether adducts, ethoxylated amide and imidazoline adducts, ethoxylated amine adducts, ethoxylated mercaptan adducts, ethoxylated condensates with alkyl phenols, ethoxylated nitrogen-based hydrophobes, ethoxylated polyoxypropylenes, polymeric silicones, fluorinated surfactants, polymerizable surfactants, and mixtures thereof.

34. The method of claim 31 wherein the sample mixture further comprises an antioxidant.

35. The method of claim 31 wherein the surfactant is present in an amount of at least about 0.5 wt-%.

36. The method of claim 35 wherein the surfactant is present in an amount of no greater than about 20 wt-%.

37. The method of claim 31 wherein the sample mixture further comprises a buffer.

38. The method of claim 31 wherein the sample mixture further comprises a dinucleotide triphosphate.

39. The method of claim 31 wherein the sample mixture further comprises a reference dye.

40. The method of claim 31 wherein the enzyme is a polymerase or a ligase.

41. A method of conducting a thermal cycling process comprising:

providing a device comprising at least one process chamber that defines a volume for containing a sample mixture comprising a biological material, a polymerase enzyme, a near-IR dye, a nonionic surfactant, and a dinucleotide triphosphate;

delivering electromagnetic energy to the process chamber to raise the temperature of the sample material in the process chamber, wherein the dye converts the electromagnetic energy into thermal energy;

wherein the surfactant inhibits interaction between the enzyme and the dye.

42. The method of claim 41 further comprising cooling the sample mixture and reheating the sample mixture in a thermal cycling process.

43. The method of claim 42 wherein the thermal cycling process comprises at least about 25 cycles.

44. The method of claim 42 wherein the thermal cycling process comprises heating between a temperature of about 50°C and about 95°C.

45. A method of denaturing hydrogen-bonded molecules, the method comprising:

providing a sample mixture comprising hydrogen-bonded molecules, an enzyme, an effective amount of a surfactant selected from the group of a nonionic surfactant, a zwitterionic surfactant, and a mixture thereof, and a dye at a first temperature; and

directly heating the sample mixture to a second temperature higher than the first temperature effective to denature the hydrogen-bonded molecules;

wherein the dye inactivates the enzyme in the absence of the surfactant and the surfactant inhibits the inactivation.

46. (NEW) A device for use in thermal processing, the device comprising:

a plurality of process chambers, each of the process chambers defining a volume for containing a sample mixture;

a valve located between selected pairs of the plurality of process chambers; and

wherein the sample mixture comprises an enzyme, a dye, and an effective amount of a surfactant from the group of a nonionic surfactant, a zwitterionic surfactant, and a mixture thereof, wherein the dye inactivates the enzyme in the absence of the surfactant, and the surfactant inhibits such interaction.

47. (NEW) The device of claim 46 wherein the valve comprises an impermeable disc distinct from a substrate.

48. (NEW) A method of conducting a thermal cycling process comprising:

providing a device comprising a plurality of process chambers, each of the process chambers defining a volume for containing a sample mixture;

providing a sample mixture in at least some of the process chambers;

delivering electromagnetic energy to the process chambers to raise the temperature of the sample mixture in the process chambers;

rotating the device about an axis of rotation while delivering the electromagnetic energy, wherein the temperature of the sample mixture in the process chambers is controlled as the substrate rotates; and

wherein the sample mixture comprises an enzyme, a dye, and an effective amount of a surfactant from the group of a nonionic surfactant, a zwitterionic surfactant, and

a mixture thereof, wherein the dye inactivates the enzyme in the absence of the surfactant, and the surfactant inhibits such interaction.

49. (NEW) A method of processing sample material comprising:

providing a device comprising a plurality of process chamber arrays, each of the process chamber arrays comprising a loading chamber, a first process chamber, and a second process chamber;

providing a sample mixture in the loading chamber of at least one of the process chamber arrays;

moving the sample mixture from the loading chamber into the first process chamber by rotating the device;

controlling the temperature of the sample mixture in the first process chamber by rotating the device about an axis of rotation while delivering electromagnetic energy to the first process chamber;

moving the sample mixture from the first process chamber to the second process chamber by rotating the device;

controlling the temperature of the sample mixture in the second process chamber by rotating the device about an axis of rotation while delivering electromagnetic energy to the second process chamber; and

wherein the sample mixture comprises an enzyme, a dye, and an effective amount of a surfactant from the group of a nonionic surfactant, a zwitterionic surfactant, and a mixture thereof, wherein the dye inactivates the enzyme in the absence of the surfactant, and the surfactant inhibits such interaction.

50. (NEW) The method of claim 49, wherein the process chamber arrays comprise a valve located between the first process chamber and the second process chamber, and wherein

the method further comprises opening the valve to move the sample mixture from the first process chamber to the second process chamber.